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(54) Title: HUMAN POTASSIUM CHANNEL 1 AND 2 PROTEINS

(57) Abstract

Disclosed are human K^+ channel polypeptides and DNA (RNA) encoding such K^+ channel polypeptides. Also provided is a procedure for producing such polypeptides by recombinant techniques. Agonists for such K^+ channel polypeptides are also disclosed. Such agonists may be used to treat epilepsy, stroke, hypertension, asthma, Parkinson's disease, schizophrenia, anxiety, depression and neurodegeneration. Also disclosed are antagonists against such polypeptides which may be used to treat AIDS, SLE, diabetes, multiple sclerosis and cancer.

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HUMAN POTASSIUM CHANNEL 1 AND 2 PROTEINS

This invention relates to newly identified polynucleotides, polypeptides encoded by such polynucleotides, the use of such polynucleotides and polypeptides, as well as the production of such polynucleotides and polypeptides. More particularly, the polypeptides of the present invention are human potassium channel proteins sometimes hereinafter referred to as a "K^+ channel 1 and 2 polypeptides." The invention also relates to inhibiting the action of such polypeptides.

Potassium channels probably form the most diverse group of ion channels, and are essential to the control of the excitability of nerve and muscle. Some potassium channels open in response to a depolarization of the membrane, others to a hyperpolarization or an increase in intracellular calcium. Some can also be regulated by the binding of a transmitter and by intracellular kinases, GTP-binding proteins or other second messengers.

Potassium channels are a heterogeneous group of ion channels that are similar in their ability to select for potassium over other ions, but differ in details of activation, inactivation and kinetics (Latorre, R. and Miller, C., J. Memb. Biol., 7:11-30, (1983)). They

contribute significantly to several physiological functions, for example, action potential r polarization, cardiac pacemaking, neuron bursting, and possibly learning and memory (Hodgkin, A.L. and Huxley, A.F., J. Physiol. 117:500-544 (1952)).

The molecular basis for potassium channel function has been greatly clarified by molecular cloning in the Drosophila family members of potassium channels, designated *Shaker, Shaw, Shal,* and *Shad* (Tempel, B.L. et al., Science, 237:770-775 (1987)). Mammalian homologs for all four of these potassium channels have been cloned, (Tempel, B.L. et al., Nature, 332:837-839 (1988)). Subtypes of Drosophila potassium channels have been identified. The subtypes in Drosophila are largely derived by alternative splicing, (Schwartz, T.L. et al., Nature, 331:137-142 (1988)), whereas subtypes of mammalian potassium channels generally represent distinct genes, although splicing occurs as well. Th biophysical properties of these channels can vary with only small alterations in the amino acid sequence, the principal differentiation being between slowly inactivating, "delay d rectifier" channels and rapidly inactivating, A-typ channels, (Wei, A. et al., Science, 248:599-603 (1990)). Mammalian homologs of Drosophila potassium channels may display either the same or different biophysical properties.

Potassium channels are involved in normal cellular homeostasis and are associated with a variety of diseas states and immune responses. Diseases believed to have a particular association with sodium, calcium and potassium channels include autoimmune diseases and other proliferative disorders such as cancers. Autoimmune diseases includ rheumatoid arthritis, type-1 diabetes mellitus, multiple sclerosis, myasthenia gravis, systematic lupus erythematosus, Sjogren's syndrome, mixed connective tissue disease among others.

S veral classes of potassium channels are involved in maintaining membrane potential and r gulating c ll volume in diverse cell typ s, as well as modulating electrical excitability in the nervous system (Lewis, R.S. and Cahalan, M.D., Science, 239:771-775 (1988)). Potassium channels have been shown to control the repolarization phase of action potentials and the pattern of firing neurons and other cells. Potassium currents have been shown to be more diverse than sodium or calcium currents, and also play a central role in determining the way a cell responds to an external stimulus. For instance, the rate of adaptation or delay with which a neuron responds to synaptic input is strongly determined by the presence of different classes of potassium channels. The molecular mechanisms generating potassium channel diversity are best understood in the *Shaker* locus from Drosophila which contains 21 exons spanning 130 kb and generates four different potassium channel proteins through alternative splicing of a single primary transcript, (DeCoursey, T.E. et al., J. Gen. Physiol. 89:379-404 (1987)). Expression of these cDNAs in *Xenopus* oocytes gives rise to voltage-dependent potassium currents with distinct physiological properties. The related Drosophila potassium channel gene *Shab* also exhibits alternative splicing of a primary transcript giving rise to two distinct proteins (McKinnon, D., and Ceredig, R., J. Exp. Med., 164:1846-1861 (1986)).

PCT Application No. WO 92/02634 discloses the n potassium channel expression product of the MK3 gene or a functionally bioactive equivalent thereof and its uses, particularly in combination with identifying immune responses and materials modulating or blocking the same.

A novel potassium channel with unique localizations in the mammalian brain has been identified, cloned and sequenced and has been designated *cdrk*, utilizing a cDNA library prepared from circumvallate papillae of the rat tongue. The *cdrk* channel appears to be a member of the *Shab's* subfamily,

most closely resembling *cdrk1*. The *cdrk* channel may b important in a variety of excitable tissues, (Hwang, P.M., *et al.*, Neuron, 8:473-481 (1992)).

Multiple potassium channel components have been produced by alternative splicing at the *Shaker* locus in *Drosophila*, (Schwarz, T.L., *et al.*, Nature, 331-137-142 (1988)).

Members of the RCK potassium channel family have been differentially expressed in the rat nervous system. mRNA'S encoding four members of the RCK potassium channel family, named RCK1, RCK3, RCK4 and RCK5 have been analyzed by RNA blot hybridization experiments using specific RNA probes, (Beckh, S. and Pongs, O., The EMBO Journal, 9:777-782 (1990)).

In accordance with one aspect of the present invention, there are provided novel mature polypeptides which are K^+ channel proteins, as well as fragments, analogs and derivatives thereof. The polypeptides of the present invention are of human origin.

In accordance with another aspect of the present invention, there are provided polynucleotides (DNA or RNA) which encode such polypeptides.

In accordance with yet a further aspect of the present invention, there is provided a process for producing such polypeptides by recombinant techniques.

In accordance with yet a further aspect of the present invention, there are provided agonists for the K^+ channel polypeptides which may be used for therapeutic purposes, for example, for treating hypertension, epilepsy, stroke, asthma, parkinson's disease, schizophrenia, anxiety, depression and neurodegeneration.

In accordance with yet a further aspect of the present invention, there are provided antibodies against such polypeptides which may used as part of a diagnostic assay for detecting autoimmune diseases and cancers.

In accordance with yet another asp ct of the present invention, ther ar provided antagonist/inhibitors to such polypeptides, which may be us d to inhibit the action of such polypeptides, for example, in the treatment of migraine headaches, autoimmune diseases, cancer and graft rejection.

These and other aspects of the present invention should be apparent to those skilled in the art from the teachings herein.

The following drawings are illustrative of embodiments of the invention and are not meant to limit the scope of the invention as encompassed by the claims.

Fig. 1 shows the cDNA sequence and deduced amino acid sequence for the putative mature K^+ channel 1 protein. The standard one-letter abbreviation for amino acids is used.

Fig. 2 shows the cDNA sequence and deduced amino acid sequence for the putative mature K^+ channel 2 protein.

Fig. 3 shows the amino acid homology between K^+ channel 2 protein (top) and Human DRK1 protein (bottom).

In accordance with an aspect of the present invention, there are provided isolated nucleic acids (polynucleotides) which encode for the mature K^+ channel 1 polypeptide having the deduced amino acid sequence of Figure 1 or for the mature polypeptide encoded by the cDNA of the clone deposited as ATCC Deposit No. 75700 on March 4, 1994.

In accordance with another aspect of the present invention, there are provided isolated nucleic acids which encode for the mature K^+ channel 2 polypeptide having the deduced amino acid sequence of Figure 2 or for the mature polypeptide encoded by the cDNA of the clone deposited as ATCC Deposit No. 75830 on July 15, 1994.

Polynucleotides encoding the polypeptides of the present invention may be obtained from brain, skeletal muscle and placental tissues. The polynucleotides of this invention were discovered in a cDNA library derived from human brain.

Th y ar structurally related to the K+ channel gen family. K^+ chann l 1 polyp ptide contains an op n reading frame encoding a polypeptide of approximately 513 amino acid residues. The polypeptide exhibits the highest degr e homology to drk1 protein with approximately 40% identity and 65% similarity over a 400 amino acid stretch.

Polynucleotides encoding the K^+ channel 2 polypeptides of the present invention were discovered in a cDNA library derived from human brain. They are structurally related to the K^+ channel gene family. K^+ channel 2 polypeptide contains an open reading frame encoding a polypeptide of approximately 494 amino acid residues. The polypeptide exhibits the highest degree of homology to human DRK1 protein with approximately 40 % identity and 66 % similarity over a 488 amino acid stretch.

The polynucleotides of the present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequence which encodes the mature polypeptides may b identical to the coding sequence shown in Figures 1 and 2 or that of the deposited clone or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same mature polypeptides as the DNA of Figures 1 and 2 or the deposited cDNA(s).

The polynucleotides which encode the mature polypeptides of Figures 1 and 2 or the mature polypeptides encoded by the deposited cDNA(s) may include: only the coding sequence for the mature polypeptides; the coding sequence for the mature polypeptides and additional coding sequence such as a leader or secretory sequence; the coding sequence for the matur polypeptides (and optionally additional coding sequence) and

non-coding sequ nce, such as introns or non-coding sequence 5' and/or 3' of the coding sequence for the mature polypeptid s.

Thus, the term "polynucleotide encoding a polypeptide" encompasses a polynucleotide which includes only coding sequence for the polypeptide as well as a polynucleotide which includes additional coding and/or non-coding sequence.

The present invention further relates to variants of the hereinabove described polynucleotides which encode fragments, analogs and derivatives of the polypeptides having the deduced amino acid sequences of Figures 1 and 2 or the polypeptides encoded by the cDNA of the deposited clones. The variants of the polynucleotides may be naturally occurring allelic variants of the polynucleotides or non-naturally occurring variants of the polynucleotides.

Thus, the present invention includes polynucleotides encoding the same mature polypeptides as shown in Figures 1 and 2 or the same mature polypeptides encoded by the cDNA of the deposited clones as well as variants of such polynucleotides which variants encode for a fragment, derivative or analog of the polypeptides of Figures 1 and 2 or the polypeptides encoded by the cDNA of the deposited clones. Such nucleotide variants include deletion variants, substitution variants and addition or insertion variants.

As hereinabove indicated, the polynucleotides may have a coding sequence which is a naturally occurring allelic variant of the coding sequences shown in Figures 1 and 2 or of the coding sequence of the deposited clones. As known in the art, an allelic variant is an alternate form of a polynucleotide sequence which may have a substitution, deletion or addition of one or more nucleotides, which does not substantially alter the function of the encoded polypeptide.

The polynucleotides of the present invention may also have the coding sequence fused in frame to a marker sequence

which allows for purification of the polyp ptides of the pr sent invention. The marker s quence may be a h xa-histidine tag supplied by a pQE-9 vector to provid for purification of the mature polypeptides fused to the marker in the case of a bacterial host, or, for example, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. COS-7 cells, is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, I., et al., Cell, 37:767 (1984)).

The present invention further relates to polynucleotides which hybridize to the hereinabove-described sequences if there is at least 50% and preferably 70% identity between the sequences. The present invention particularly relates to polynucleotides which hybridize under stringent conditions to the hereinabove-describ d polynucleotides . As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences. The polynucleotides which hybridize to the hereinabove described polynucleotides in a preferr d embodiment encode polypeptides which retain substantially the same biological function or activity as the mature polypeptides encoded by the cDNA of Figures 1 and 2 or the deposited cDNA(s).

The deposit(s) referred to herein will be maintain d under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for purposes of Patent Procedure. These deposits are provided merely as convenience to those of skill in the art and are not an admission that a deposit is required under 35 U.S.C. §112. The sequence of the polynucleotides contained in th deposited materials, as well as the amino acid sequence of the polypeptides encoded thereby, are incorporated herein by reference and are controlling in the event of any conflict with any description of sequences herein. A license may be

required to make, use or sell the deposit d materials, and no such license is hereby granted.

The present invention further relat s to K^+ channel polypeptides which have the deduced amino acid sequences of Figures 1 and 2 or which have the amino acid sequence encoded by the deposited cDNA(s), as well as fragments, analogs and derivatives of such polypeptides.

The terms "fragment," "derivative" and "analog" when referring to the polypeptides of Figures 1 and 2 or that encoded by the deposited cDNA(s), means polypeptides which either retain essentially the same biological function or activity as such polypeptides, or retain the ability to bind the ligand of the K^+ channel polypeptide, however, are a soluble form of such polypeptide and, therefore, elicit no function.

The polypeptides of the present invention may be a recombinant polypeptide, a natural polypeptide or a synthetic polypeptide, preferably a recombinant polypeptide.

The fragment, derivative or analog of the polypeptides of Figures 1 and 2 or that encoded by the deposited cDNA may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptides are fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptides, such as a leader or secretory sequence or a sequence which is employed for purification of the mature polypeptides. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

The polypeptid s and polynucleotides of the present invention ar pr f rably provid d in an isolated form, and preferably are purified to homogeneity.

The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment.

The present invention also relates to vectors which include polynucleotides of the present invention, host cells which are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques.

Host cells are genetically engineered (transduced or transformed or transfected) with the vectors of this invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the K^+ channel protein genes. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

The polynucleotides of the present invention may be employed for producing polypeptides by recombinant techniques. Thus, for example, the polynucleotide may b included in any one of a variety of expression vectors for

expr ssing a polypeptid . Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector may be used as long as it is replicable and viable in the host.

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and others are deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, there may be mentioned: LTR or SV40 promoter, the E. coli. lac or trp, the phage lambda P_L promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression.

In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in E. coli.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the protein.

As repr s ntative exampl s of appropriate hosts, there may be mentioned: bacterial c lls, such as *E. coli*, *Streptomyc s*, *Salmonella typhimurium*; fungal cells, such as yeast; insect cells such as *Drosophila* and *Sf9*; animal cells such as CHO, COS, HEK 293 or Bowes melanoma; plant cells, etc. The selection of an appropriate host is deemed to b within the scope of those skilled in the art from th teachings herein.

More particularly, the present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. The following vectors are provided by way of example. Bacterial: pQE70, pQE60, pQE-9 (Qiagen), pbs, pD10, phagescript, psiX174, pbluescript SK, pbsks, pNH8A, pNH16a, pNH18A, pNH46A (Stratagene); ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia). Eukaryotic: pWLNEO, pSV2CAT, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia). However, any other plasmid or vector may be used as long as they are replicable and viable in the host.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors ar PKK232-8 and PCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P_R, P_L and trp. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriat

vector and promoter is well within the level of ordinary skill in the art.

In a further embodiment, the present invention relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation. (Davis, L., Dibner, M., Battey, I., Basic Methods in Molecular Biology, (1986)).

The constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the polypeptides of the invention can be synthetically produced by conventional peptide synthesizers.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), the disclosure of which is hereby incorporated by reference.

Transcription of the DNA encoding the polypeptides of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples including the SV40 enhancer on the late side of the replication origin bp 100 to 270, a cytomegalovirus early

promoter enhancer, the polyoma enhancer on the lat side of the replication origin, and adenovirus enhanc rs.

Generally, recombinant xpr ssion vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of E. coli and S. cerevisiae TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), α-factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phas with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitabl prokaryotic hosts for transformation include E. coli, Bacillus subtilis, Salmonella typhimurium and various species within the genera Pseudomonas, Streptomyces, and Staphylococcus, although others may also be employed as a matter of choice.

As a representative but nonlimiting example, useful expression vectors for bacterial use can comprise a

selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic el ments of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period.

Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification. Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, such methods are well know to those skilled in the art.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell, 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5′ flanking nontranscribed sequences. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

The K^+ channel polypeptides can be recovered and purified from recombinant cell cultures by methods including ammonium sulfat or ethanol pr cipitation, acid extraction, anion or cation exchange chromatography, phosphocellulos chromatography, hydrophobic interaction chromatography, affinity chromatography hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

The polypeptides of the present invention may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniqu s from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. Polypeptides of the invention may also include an initial methionine amino acid residue.

The present invention relates to an assay for identifying molecules which have a modulating effect, eg. drugs, agonists or antagonists, on the K^+ channel polypeptides of the present invention. Such an assay comprises the steps of providing an expression system that produces a functional K^+ channel expression product encoded by the DNA of the present invention, contacting th expression system or the product of the expression system with one or more molecules to determine its modulating effect on the bioactivity of the product and selecting from th molecules a candidate capable of modulating K^+ chann l expression.

Antagonists to the K^+ channel openers, including those identified by the method above, are K^+ channel openers, which

incr ase K^+ ion flux and, th refore, are useful for treating epil psy, stroke, hypertension, asthma, Parkinson's dis ase, schizophrenia, anxiety, depression and neurodegeneration. While applicant does not wish to limit the scientific reasoning behind these therapeutic uses, the high degree of localization of K^+ channel proteins in the brain, nervous system and myocardium, K^+ ion flux through the K+ channels of the present invention provides an ion balance and a concurrent therapeutic result.

Potential antagonists to the K^+ channel polypeptides of the present invention include an antibody against the K^+ channel polypeptides, or in some cases, an oligonucleotide, which bind to the K^+ channel polypeptides and alter its conformation such that K^+ ions do not pass therethrough. Soluble K^+ Channel 1 polypeptides may also be used as antagonists by administering them into circulation to bind free K^+ ions and, therefore, reduce their concentration *in vivo*.

Potential antagonists also include antisense constructs produced by antisense technology. Antisense technology controls gene expression through triple-helix formation, etc. The number of K^+ Channels may be reduced through antisense technology, which controls gene expression through triple-helix formation or antisense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion of the polynucleotide sequence, which encodes for the mature polypeptides of the present invention, is used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription (triple helix -see Lee et al., Nucl. Acids Res., 6:3073 (1979); Cooney et al, Science, 241:456 (1988); and Dervan et al., Science, 251: 1360 (1991)), thereby

preventing transcription and the production of the K^+ channel polypeptides. The antis nse RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into the K^+ channel polypeptides (antisense - Okano, J. Neurochem., 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). The antisense constructs can be delivered to cells by procedures known in the art such that the antisense RNA or DNA may be expressed *in vivo*.

Another example of potential antagonists include a small molecule which binds to and occupies the opening in the K^+ channel polypeptide thereby not allowing K^+ ions to pass therethrough, such that normal biological activity is prevented. Examples of small molecules include but are not limited to small peptides or peptide-like molecules.

The antagonists which exert their effect upon the K^+ channel polypeptides may be used to treat autoimmune diseases which result from abnormal cells of the immune system destroying target tissues, either by direct killing or by producing autoantibodies. In a normal immune response the n channel type of K^+ channel proteins are increased upwards of ten fold in normal T cells. Accordingly, the antagonist/inhibitors may be employed to treat autoimmune diseases such as AIDS, SLE, diabetes mellitus, multipl sclerosis and lymphocyte-mediated immune reaction against transplantation antigens. The antagonist/inhibitors may also be used to treat cell-proliferative conditions, such as cancer and tumoricity, which have a similar association with immunologic factors. The antagonist/inhibitors may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as hereinafter described.

The agonists or antagonists of the K^+ channel polypeptides of the present invention may be employed in combination with a suitable pharmaceutical carrier to

comprise a pharmaceutical composition. Such compositions comprise a th rapeutically effective amount of the agonist or antagonist, as th case may be, and a pharmac utically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The formulation should suit the mode of administration.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptides of the present invention may be employed in conjunction with other therapeutic compounds.

The pharmaceutical compositions may be administered in a convenient manner such as by the intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes. The compositions are administered in an amount which is effective for treating and/or prophylaxis of the specific indication. In general, the compositions will be administered in an amount of at least about 10 μg/kg body weight and in most cases they will be administered in an amount not in excess of about 8 mg/Kg body weight per day. In most cases, the dosage is from about 10 μg/kg to about 1 mg/kg body weight daily, taking into account the routes of administration, symptoms, etc.

The agonists or antagonists which are polypeptides may be employed in accordance with the present invention by expression of such polypeptides *in vivo*, which is often referred to as "gene therapy."

Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) encoding a

polypeptide x vivo, with the engin ered cells th n being pr vided to a patient to be treat d with the polyp ptide. Such methods ar well-known in the art. For exampl , c lls may be engineered by procedures known in the art by use of a retroviral particle containing RNA encoding a polypeptide of the present invention.

Similarly, cells may be engineered *in vivo* for expression of a polypeptide *in vivo* by, for example, procedures known in the art. As known in the art, a produc r cell for producing a retroviral particle containing RNA encoding the polypeptide of the present invention may be administered to a patient for engineering cells *in vivo* and expression of the polypeptide *in vivo*. These and oth r methods for administering a polypeptide of the present invention by such method should be apparent to those skilled in the art from the teachings of the present invention. For example, the expression vehicle for engineering cells may be other than a retrovirus, for example, an adenovirus which may be used to engineer cells in vivo after combination with a suitable delivery vehicle.

The sequences of the present invention are also valuabl for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the cDNA is used to rapidly select primers that do not span more than one exon in the genomic

DNA, thus complicating the amplification process. These prim rs are then used for PCR scr ening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the primer will yield an amplified fragment.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular DNA to a particular chromosome. Using the present invention with the same oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes or pools of large genomic clones in an analogous manner. Other mapping strategies that can similarly be used to map to its chromosome include *in situ* hybridization, prescreening with labeled flow-sorted chromosomes and preselection by hybridization to construct chromosome specific-cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with cDNA as short as 500 or 600 bases; however, clones larger than 2,000 bp have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. FISH requires use of the clones from which the EST was derived, and the longer the better. For example, 2,000 bp is good, 4,000 is better, and more than 4,000 is probably not necessary to get good results a reasonable percentage of the time. For a review of this technique, see Verma et al., Human Chromosomes: a Manual of Basic Techniques, Pergamon Press, New York (1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library).

With curr nt resolution of physical mapping and genetic mapping techniques, a cDNA precis ly localized to a chromosomal region associated with the disease could be on of between 50 and 500 potential causative genes. (This assumes 1 megabase mapping resolution and one gene per 20 kb).

The polypeptides, their fragments or other derivatives, or analogs thereof, or cells expressing them can be used as an immunogen to produce antibodies thereto. These antibodies can be, for example, polyclonal or monoclonal antibodies. The present invention also includes chimeric, single chain, and humanized antibodies, as well as Fab fragments, or th product of an Fab expression library. Various procedures known in the art may be used for the production of such antibodies and fragments.

Antibodies generated against the polypeptides corresponding to a sequence of the present invention can be obtained by direct injection of the polypeptides into an animal or by administering the polypeptides to an animal, preferably a nonhuman. The antibody so obtained will then bind the polypeptides itself. In this manner, even a sequence encoding only a fragment of the polypeptides can b used to generate antibodies binding the whole nativ polypeptides. Such antibodies can then be used to isolat the polypeptide from tissue expressing that polypeptide.

For preparation of monoclonal antibodies, any techniqu which provides antibodies produced by continuous cell lin cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, 1975, Nature, 256:495-497), the trioma technique, the human B-cell hybridoma techniqu (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodi s (Cole, et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can b adapted to produce single chain antibodies to immunog nic polypeptide products of this invention.

In accordance with another aspect of the present invention, there is provided an assay for diagnosing a diseased state associated with K^+ channel expression mediated T cell activation comprising providing T cells containing K^+ channels from a test individual, identifying activated T cells from among the population of T cells and measuring the activation of the T cells relative to the total T cell population by measuring K^+ channel expression using labeling means based on a functionally bioactive product of DNA encoding the genes of the present invention. This assay may be used to detect autoimmune diseases and cancer, since T cells associated with these conditions have an elevated number of K^+ channels.

The present invention will be further described with reference to the following examples; however, it is to be understood that the present invention is not limited to such examples. All parts or amounts, unless otherwise specified, are by weight.

In order to facilitate understanding of the following examples certain frequently occurring methods and/or terms will be described.

"Plasmids" are designated by a lower case p preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

"Digestion" of DNA refers to catalytic cleavag of the DNA with a restriction enzyme that acts only at certain sequences in the DNA. The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors and other requirements were used as would be known to the ordinarily skilled artisan. For analytical purposes, typically 1 μg of plasmid or DNA fragment is used with about 2 units of enzyme in about 20 μl of buffer solution. For the purpose of isolating DNA fragments for plasmid construction, typically 5 to 50 μg of DNA are digested with 20 to 250 units of enzyme in a larger volume. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by th manufacturer. Incubation times of about 1 hour at 37°C are ordinarily used, but may vary in accordance with the supplier's instructions. After digestion the reaction is electrophoresed directly on a polyacrylamide gel to isolate the desired fragment.

Size separation of the cleaved fragments is performed using 8 percent polyacrylamide gel described by Goeddel, D. *et al.*, Nucleic Acids Res., 8:4057 (1980).

"Oligonucleotides" refers to either a single stranded polydeoxynucleotide or two complementary polydeoxynucleotide strands which may be chemically synthesized. Such synthetic oligonucleotides have no 5' phosphate and thus will not ligate to another oligonucleotide without adding a phosphat with an ATP in the presence of a kinase. A synthetic oligonucleotide will ligate to a fragment that has not been dephosphorylated.

"Ligation" refers to the process of forming phosphodiester bonds between two double stranded nucleic acid fragments (Maniatis, T., et al., Id., p. 146). Unless otherwise provided, ligation may be accomplished using known buffers and conditions with 10 units to T4 DNA ligas

("ligase") per 0.5 μg of approximately equimolar amounts of the DNA fragments to be ligated.

Unless otherwise stated, transformation was performed as described in the method of Graham, F. and Van der Eb, A., Virology, 52:456-457 (1973).

Example 1

Bacterial Expression and Purification of K^+ Channel 1 Protein

The DNA sequence encoding for the K^+ channel 1 polypeptides of the present invention, ATCC # 75700, is initially amplified using PCR oligonucleotide primers corresponding to the 5' and sequences of the processed K^+ channel 1 protein (minus the signal peptide sequence) and the vector sequences 3' to the K^+ channel protein gene. Additional nucleotides corresponding to K^+ channel 1 protein are added to the 5' and 3' sequences respectively. The 5' oligonucleotide primer has the sequence 5' GACTAAAGCTTAATGACCCTCTTACCGGG 3' contains a Hind III restriction enzyme site followed by 17 nucleotides of the coding sequence starting from the presumed terminal amino acid of the protein codon. The 3' sequence 3' GAACTTCTAGACCGCGCTCAGTCATTGTC 5' contains complementary sequences to an Xba I restriction enzyme site and is followed by 18 nucleotides of the non-coding sequence located 3' to the K^+ channel 1 protein DNA insert and to a pBluescript SK+ vector sequence located 3' to the K^+ channel 1 protein DNA insert. The restriction enzyme sites correspond to the restriction enzyme sites on the bacterial expression vector pQE-9. (Qiagen, Inc. 9259 Eton Avenue, Chatsworth, CA, 91311). pQE-9 encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an IPTG-regulatable promoter operator (P/O), a ribosome binding site (RBS), a 6-His tag and restriction enzyme sites. pQE-9 is then digested with Hind III and Xba I. The amplified sequences are ligated

into pQE-9 and are insert d in fram with th sequence encoding for the histidine tag and the RBS. Th ligation mixture is then us d to transform the E. coli strain M15/rep4 available from Qiagen under the trademark M15/rep 4 by the procedure described in Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989). M15/rep4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies were selected. Plasmid DNA is isolated and confirmed by restriction analysis. Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells ar grown to an optical density 600 ($O.D.^{600}$) of between 0.4 and 0.6. IPTG ("Isopropyl-B-D-thiogalacto pyranoside") is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells are grown an extra 3 to 4 hours. Cells are then harvested by centrifugation. The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl. After clarification, solubilized K^+ channel protein is purified from this solution by chromatography on a Nickel-Chelate column under conditions that allow for tight binding by proteins containing the 6-His tag (Hochuli, E. et al., J. Chromatography 411:177-184 (1984)). K^+ channel 1 protein is eluted from the column in 6 molar guanidine HCl pH 5.0 and for the purpose of renaturation adjusted to 3 molar guanidine HCl, 100mM sodium phosphate, 10 mmolar glutathion (reduced) and 2 mmolar glutathione (oxidized). After incubation in this solution for 12 hours the protein is dialyzed to 10 mmolar sodium phosphate.

Example 2

Cloning and expression of K+ channel 1 protein using the baculovirus expression system

The DNA sequence encoding the full length K+ channel 1 protein, ATCC # 75700, was amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene:

The 5' primer has the sequence 5' CGGGATCCCTCCATGACCCTCTTACCGGGA 3' and contains a BamH1 restriction enzyme site followed by 4 nucleotides resembling an efficient signal for the initiation of translation in eukaryotic cells (J. Mol. Biol. 1987, 196, 947-950, Kozak, M.), and just behind the first 18 nucleotides of the K+ channel 1 gene (the initiation codon for translation "ATG" is underlined).

The 3' primer has the sequence 5' CGGGATCCCGCTCAGTTATTGTCTCTGGT 3' and contains the cleavage site for the restriction endonuclease BamH1 and 18 nucleotides complementary to the 3' non-translated sequence of the K+ channel 1 gene. The amplified sequences were isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment was then digested with the endonuclease BamH1 and then purified on a 1% agarose gel using a commercially available kit ("Geneclean" BIO 101 Inc., La Jolla, Ca.). This fragment is designated F2.

The vector pRG1 (modification of pVL941 vector, discussed below) is used for the expression of the K+ channel 1 protein using the baculovirus expression system (for review see: Summers, M.D. and Smith, G.E. 1987, A manual of methods for baculovirus vectors and insect cell culture procedures, Texas Agricultural Experimental Station Bulletin No. 1555). This expression vector contains the strong polyhedrin promoter of the Autographa californica nuclear polyhedrosis virus (AcMNPV) followed by the recognition sites for the

r striction endonuclease BamH1. The polyad nylation site of the simian virus (SV)40 is used for efficient polyad nylation. For an easy s lection of recombinant viruses the beta-galactosidase gene from E.coli is inserted in the same orientation as the polyhedrin promoter followed by the polyadenylation signal of the polyhedrin gene. The polyhedrin sequences are flanked at both sides by viral sequences for the cell-mediated homologous recombination of cotransfected wild-type viral DNA. Many other baculovirus vectors could be used in place of pRG1 such as pAc373, pVL941 and pAcIM1 (Luckow, V.A. and Summers, M.D., Virology, 170:31-39).

The plasmid was digested with the restriction enzym s BamH1 and then dephosphorylated using calf intestinal phosphatase by procedures known in the art. The DNA was th n isolated from a 1% agarose gel and purified again on a 1% agarose gel. This vector DNA is designated V2.

Fragment F2 and the dephosphorylated plasmid V2 wer ligated with T4 DNA ligase. E.coli HB101 cells were then transformed and bacteria identified that contained the plasmid (pBacK+ channel 1) with the K+ channel 1 gene using the enzymes BamH1. The sequence of the cloned fragment was confirmed by DNA sequencing.

5 μg of the plasmid pBacK+ channel 1 were cotransfected with 1.0 μg of a commercially available lineariz d baculovirus ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA.) using the lipofection method (Felgner et al. Proc. Natl. Acad. Sci. USA, 84:7413-7417 (1987)).

1μg of BaculoGold™ virus DNA and 5 μg of the plasmid pBacK+ channel 1 were mixed in a sterile well of a microtiter plate containing 50 μl of serum free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards 10 μl Lipofectin plus 90 μl Grace's medium were added, mixed and incubated for 15 minutes at room temperature. Then th transfection mixture was added dropwise to the Sf9 insect

c lls (ATCC CRL 1711) se d d in a 35 mm tissue culture plate with 1ml Grace' medium without s rum. The plate was rock d back and forth to mix the n wly add d solution. The plate was then incubated for 5 hours at 27°C. After 5 hours the transfection solution was removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum was added. The plate was put back into an incubator and cultivation continued at 27°C for four days.

After four days the supernatant was collected and a plaque assay performed similar as described by Summers and Smith (supra). As a modification an agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) was used which allows an easy isolation of blue stained plaques. (A detailed description of a "plaque assay" can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10).

Four days after the serial dilution of the viruses was added to the cells, blue stained plaques were picked with the tip of an Eppendorf pipette. The agar containing the recombinant viruses was then resuspended in an Eppendorf tube containing 200 μl of Grace's medium. The agar was removed by a brief centrifugation and the supernatant containing the recombinant baculoviruses was used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes were harvested and then stored at 4°C.

Sf9 cells were grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells were infected with the recombinant baculovirus V-K+ channel 1 at a multiplicity of infection (MOI) of 2. Six hours later the medium was removed and replaced with SF900 II medium minus methionine and cysteine (Life Technologies Inc., Gaithersburg). 42 hours later 5 μCi of ^{35}S-methionine and 5 μCi ^{35}S cysteine (Amersham) were added. The cells were further incubated for 16 hours

before they were harvested by centrifugation and th labelled pr teins visualized by SDS-PAGE and autoradiography.

Example 3

Expression of Recombinant K+ channel 1 protein in COS cells

The expression of plasmid, pK+ channel 1 HA is derived from a vector pcDNAI/Amp (Invitrogen) containing: 1) SV40 origin of replication, 2) ampicillin resistance gene, 3) E.coli replication origin, 4) CMV promoter followed by a polylinker region, a SV40 intron and polyadenylation site. A DNA fragment encoding the entire K+ channel 1 protein and a HA tag fused in frame to its 3' end was cloned into the polylinker region of the vector, therefore, the recombinant protein expression is directed under the CMV promoter. The HA tag correspond to an epitope derived from the influenza hemagglutinin protein as previously described (I. Wilson, H. Niman, R. Heighten, A Cherenson, M. Connolly, and R. Lerner, 1984, Cell 37, 767). The infusion of HA tag to our target protein allows easy detection of the recombinant protein with an antibody that recognizes the HA epitope.

The plasmid construction strategy is described as follows:

The DNA sequence encoding for K+ channel 1 protein, ATCC # 75700, was constructed by PCR on the full-length gene cloned using two primers: the 5' primer 5' GTCCAAGCTTGCCACCATGACCCTCTTACCCGGA 3' contains a HindIII sit followed by 18 nucleotides of K+ channel 1 coding sequenc starting from the initiation codon; the 3' sequence 5' CTAGCTCGAGTCAAGCGTAGTCTGGGACGTCGTATGGGTAGCAGTTATTGTCTCTGGT 3' contains complementary sequences to an XhoI site, translation stop codon, HA tag and the last 15 nucleotides of the K+ channel 1 coding sequence (not including the stop codon). Therefore, the PCR product contains a HindIII site, K+ channel 1 coding sequence followed by HA tag fused in frame, a translation termination stop codon next to the HA tag, and

an Xho I site. The PCR amplified DNA fragment and the v ctor, pcDNAI/Amp, wer digested with HindIII and XhoI restriction nzymes and ligat d. The ligation mixture was transformed into E. coli strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037) the transformed culture was plated on ampicillin media plates and resistant colonies were selected. Plasmid DNA was isolated from transformants and examined by restriction analysis for the presence of the correct fragment. For expression of the recombinant K+ channel 1, COS cells were transfected with the expression vector by DEAE-DEXTRAN method. (J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989)). The expression of the K+ channel 1 HA protein was detected by radiolabelling and immunoprecipitation method. (E. Harlow, D. Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, (1988)). Cells were labelled for 8 hours with ^{35}S-cysteine two days post transfection. Culture media were then collected and cells were lysed with detergent (RIPA buffer (150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50mM Tris, pH 7.5). (Wilson, I. et al., Id. 37:767 (1984)). Both cell lysate and culture media were precipitated with a HA specific monoclonal antibody. Proteins precipitated were analyzed on 15% SDS-PAGE gels.

Example 4

Cloning and expression of K+ channel 2 protein using the baculovirus expression system

The DNA sequence encoding the full length K+ channel 2 protein, ATCC # 75830, was amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene:

The 5' primer has the sequence 5' CGGATCCCTCCATGGACGGGTCCGGGGAG 3' and contains a BamH1 restriction enzyme site followed by 4 nucleotides resembling

an ffici nt signal for th initiation of translation in eukaryotic cells (J. Mol. Biol. 1987, 196, 947-950, Kozak, M.), and just b hind the first 18 nucleotid s of the K+ channel 2 gene (the initiation codon for translation "ATG" is underlined).

The 3' primer has the sequence 5' CGGGATCCCGCTCACTTGCAACTCTGGAG 3' and contains the cleavage site for the restriction endonuclease BamH1 and 18 nucleotides complementary to the 3' non-translated sequenc of the K+ channel 2 gene. The amplified sequences were isolated from a 1% agarose gel using a commercially availabl kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment was then digested with the endonuclease BamH1 and then purified again on a 1% agarose gel. This fragment is designated F2.

The vector pRG1 (modification of pVL941 vector, discussed below) is used for the expression of the K+ channel 2 protein using the baculovirus expression system (for review see: Summers, M.D. and Smith, G.E. 1987, A manual of methods for baculovirus vectors and insect cell culture procedures, Texas Agricultural Experimental Station Bulletin No. 1555). This expression vector contains the strong polyhedrin promoter of the Autographa californica nuclear polyhedrosis virus (AcMNPV) followed by the recognition sites for th restriction endonuclease BamH1. The polyadenylation site of the simian virus (SV)40 is used for efficient polyadenylation. For an easy selection of recombinant viruses the beta-galactosidase gene from E.coli is insert d in the same orientation as the polyhedrin promoter follow d by the polyadenylation signal of the polyhedrin gene. Th polyhedrin sequences are flanked at both sides by viral sequences for the cell-mediated homologous recombination of cotransfected wild-type viral DNA. Many other baculovirus vectors could be used in place of pRG1 such as pAc373, pVL941

and pAcIM1 (Luckow, V.A. and Summers, M.D., Virology, 170:31-39).

The plasmid was dig sted with the restriction enzymes BamH1 and then dephosphorylated using calf intestinal phosphatase by procedures known in the art. The DNA was then isolated from a 1% agarose gel and purified again on a 1% agarose gel. This vector DNA is designated V2.

Fragment F2 and the dephosphorylated plasmid V2 were ligated with T4 DNA ligase. E.coli HB101 cells were then transformed and bacteria identified that contained the plasmid (pBacK+ channel 2) with the K+ channel 2 gene using the enzymes BamH1. The sequence of the cloned fragment was confirmed by DNA sequencing.

5 μg of the plasmid pBacK+ channel 2 were cotransfected with 1.0 μg of a commercially available linearized baculovirus ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA.) using the lipofection method (Felgner et al. Proc. Natl. Acad. Sci. USA, 84:7413-7417 (1987)).

1μg of BaculoGold™ virus DNA and 5 μg of the plasmid pBacK+ channel 2 were mixed in a sterile well of a microtiter plate containing 50 μl of serum free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards 10 μl Lipofectin plus 90 μl Grace's medium were added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture was added dropwise to the Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1ml Grace' medium without serum. The plate was rocked back and forth to mix the newly added solution. The plate was then incubated for 5 hours at 27°C. After 5 hours the transfection solution was removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum was added. The plate was put back into an incubator and cultivation continued at 27°C for four days.

After four days the supernatant was collected and a plaque assay performed similar as described by Summers and

Smith (supra). As a modification an agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) was used which allows an easy isolation of blue stained plaques. (A detailed description of a "plaque assay" can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10).

Four days after the serial dilution of the viruses was added to the cells, blue stained plaques were picked with the tip of an Eppendorf pipette. The agar containing the recombinant viruses was then resuspended in an Eppendorf tube containing 200 μl of Grace's medium. The agar was removed by a brief centrifugation and the supernatant containing the recombinant baculoviruses was used to infect Sf9 cells seed d in 35 mm dishes. Four days later the supernatants of these culture dishes were harvested and then stored at 4°C.

Sf9 cells were grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells were infected with the recombinant baculovirus V-K+ channel 2 at a multiplicity of infection (MOI) of 2. Six hours later the medium was removed and replaced with SF900 II medium minus methionine and cysteine (Life Technologies Inc., Gaithersburg). 42 hours later 5 μCi of ^{35}S-methionine and 5 μCi ^{35}S cysteine (Amersham) were added. The cells were further incubated for 16 hours before they were harvested by centrifugation and the labelled proteins visualized by SDS-PAGE and autoradiography.

Example 5

Expression of Recombinant K+ channel 2 protein in COS cells

The expression of plasmid, pK+ channel 2 HA is deriv d from a vector pcDNAI/Amp (Invitrogen) containing: 1) SV40 origin of replication, 2) ampicillin resistance gene, 3) E.coli replication origin, 4) CMV promoter followed by a polylinker region, a SV40 intron and polyadenylation sit . A DNA fragment encoding the entire K+ channel 2 protein and

a HA tag fused in frame to its 3' end was cloned into the polylinker region of th v ctor, th r fore, th recombinant pr tein xpression is directed under th CMV pr moter. Th HA tag correspond to an epitope derived from the influenza hemagglutinin protein as previously described (I. Wilson, H. Niman, R. Heighten, A Cherenson, M. Connolly, and R. Lerner, 1984, Cell 37, 767). The infusion of HA tag to our target protein allows easy detection of the recombinant protein with an antibody that recognizes the HA epitope.

The plasmid construction strategy is described as follows:

The DNA sequence encoding for K+ channel 2 protein, ATCC # 75830, was constructed by PCR on the full-length gene cloned using two primers: the 5' primer 5' GTCCAAGCTTGCCACCATGGACGGGTCCGGGGAG 3' contains a HindIII site followed by 18 nucleotides of K+ channel 2 coding sequence starting from the initiation codon; the 3' sequence 5' CTAGCTCGAGTCAAGCGTAGTCTGGGACGTCGTATGGGTAGCACTTGCAACTCTGGAGCCG 3' contains complementary sequences to an XhoI site, translation stop codon, HA tag and the last 18 nucleotides of the K+ channel 2 coding sequence (not including the stop codon). Therefore, the PCR product contains a HindIII site, K+ channel 2 coding sequence followed by HA tag fused in frame, a translation termination stop codon next to the HA tag, and an Xho I site. The PCR amplified DNA fragment and the vector, pcDNAI/Amp, were digested with HindIII and XhoI restriction enzymes and ligated. The ligation mixture was transformed into E. coli strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037) the transformed culture was plated on ampicillin media plates and resistant colonies were selected. Plasmid DNA was isolated from transformants and examined by restriction analysis for the presence of the correct fragment. For expression of the recombinant K+ channel 2, COS cells were transfected with the expression vector by

DEAE-DEXTRAN method. (J. Sambrook, E. Fritsch, T. Maniatis, Mol cular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989)). The expression f th K+ channel 2 HA protein was detected by radiolabelling and immunoprecipitation method. (E. Harlow, D. Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, (1988)). Cells were labelled for 8 hours with ^{35}S-cysteine two days post transfection. Culture media were then collected and cells were lysed with detergent (RIPA buffer (150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50mM Tris, pH 7.5). (Wilson, I. et al., Id. 37:767 (1984)). Both cell lysate and culture media were precipitated with a HA specific monoclonal antibody. Proteins precipitated were analyzed on 15% SDS-PAGE gels.

Numerous modifications and variations of the present invention are possible in light of the abov teachings and, therefore, within the scope of the append d claims, the invention may be practiced otherwise than as particularly described.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: LI, ET AL.

(ii) TITLE OF INVENTION: Potassium Channel Protein 1 and 2

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
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(E) COUNTRY: USA
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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 3.5 INCH DISKETTE
(B) COMPUTER: IBM PS/2
(C) OPERATING SYSTEM: MS-DOS
(D) SOFTWARE: WORD PERFECT 5.1

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: SUBMITTED HEREWITH
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: FERRARO, GREGORY D.

(B) REGISTRATION NUMBER: 36,134
(C) REFERENCE/DOCKET NUMBER: 325800-105

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 201-994-1700
(B) TELEFAX: 201-994-1744

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 2127 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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ACAAAAGCTG GAGCTCCACC GCGGTGCGGC CGCTCTAGAA CTAGTGGATC CCCCGGGCTG   60
CAGGGGCTCC GAGGGCGGGA GCTGAGCCGG GCCCCGGGAC CGAAGTTTGG CGGCGGCTCC  120
GGGAGGCAGA GCGGGCTCCC CGGGCGACTT CCAGGCCCCT CTCGCGTCCT CGCCCCGGAC  180
CCGTGGGCAG TCGGGGGGGA CGGAAGCCGC GGCCGGGCCA ACTCCGAGGC GGGGACGCCG  240
CGACGGGAAC TTGAGGCCCG AGAGGGATGT GAAGGCCCAA AATGACCCTC TTACCGGGAG  300
ACAATTCTGA CTACGACTAC AGCGCGCTGA GCTGCACCTC GGACGCCTCC TTCCACCCGG  360
CCTTCCTCCC GCAGCGCCAG GCCATCAAGG GCGCGTTCTA CCGCCGGGCG CAGCGGCTGC  420
GGCCGCAGGA TGAGCCCCGC CAGGGCTGTC AGCCCGAGGA CCGCCGCCGT CGGATCATCA  480
TCAACGTAGG CGGCATCAAG TACTCGCTGC CCTGGACCAC GCTGGACGAG TTCCCGCTGA  540
CGCGCCTGGG CCAGCTCAAG GCCTGCACCA ACTTCGACGA CATCCTCAAC GTGTGCGATG  600
ACTACGACGT CACCTGCAAC GAGTTCTTCT TCGACCGCAA CCCGGGGGCC TTCGGCACTA  660
TCCTGACCTT CCTGCGCGCG GGCAAGCTGC GGCTGCTGCG CGAGATGTGC GCGCTGTCCT  720
TCCAGGAGGA GCTGCTGTAC TGGGGCATCG CGGAGGACCA CCTGGACGGC TGCTGCAAGC  780
GCCGCTACCT GCAGAAGATT GAGGAGTTCG CGGAGATGGT GGAGCGGGAG GAAGAGGACG  840
ACGCGCTGGA CAGCGAGGGC CGCGACAGCG AGGGCCCGGC CGAGGGCGAG GGCCGCCTGG  900
GGCGCTGCAT GCGGCGACTG CGCGACATGG TGGAGAGGCC GCACTCGGGG CTGCCTGGCA  960
AGGTGTTCGC CTGCCTGTCG GTGCTCTTCG TGACCGTCAC CGCCGTCAAC CTCTCCGTCA 1020
GCACCTTGCC CAGCCTGAGG GAGGAGGAGG AGCAGGGCCA CTGTTCCCAG ATGTGCCACA 1080
ACGTCTTCAT CGTGGAGTCG GTGTGCGTGG GCTGGTTCTC CCTGGAGTTC CTCCTGCGGC 1140
TCATTCAGGC GCCCAGCAAG TTCGCCTTCC TGCGGAGCCC GCTGACGCTG ATCGACCTGG 1200
```

TGGCCATCCT GCCCTACTAC ATCACGCTGC TGGTGGACGG CGCCGCCGCA GGCCGTCGCA 1260
AGCCCGGCGC GGGCAACAGC TACCTGGACA AGGTGGGGCT GGTGCTGCGC GTGCTGCGGG 1320
CGCTGCGCAT CCTGTACGTG ATGCGCCTGG CGCGCCACTC CCTGGGGCTG CA ACGCTGG 1380
GGCTCACGGC CCGCCGCTGC ACCCGCGAGT TCGGGCTCCT GCTGCTCTTC CTCTGCGTGG 1440
CCATCGCCCT CTTCGCGCCC CTGCTCTACG TCATCGAGAA CGAGATGGCC GACAGCCCCG 1500
AGTTCACCAG CATCCCTGCC TGCTACTGGT GGGCTGTCAT CACCATGACG ACGGTGGACT 1560
ATGGCGACAT GGTCCCCAGG AGCACCCCGG GCCAGGTAGT GGCCCTGAGC AGCATCCTGA 1620
GCGGCATCCT GCTCATGGCC TTCCCAGTCA CCTCCATCTT CCACACCTTC TCCCCCTCCT 1680
ACCTGGAGCT CAAACAGGAG CAAGAGAGGG TGATGTTCCG GAGGGCGCAG TTCCTCATCA 1740
AAACCAAGTC GCAGCTGAGC GTGTCCCAGG ACAGTGACAT CTTGTTCGGA AGTGCCTCCT 1800
CGGACACCAG AGACAATAAC TGAGCGCGGA GGACACGCCT GCCCTGCCTG CCATCTGTGG 1860
CCCGAAGCCA TTGCCATCCA CTGCAGACGC CTGGAGAGGG ACAGGCCGCT TCCGAGTGCA 1920
GTCCTGGCGC AGCACCGACT CCCACGCACC CGGGGAAGGA CACCCTCACT CCCACACCCC 1980
GGGAAGAACA CTAGAACATC AGCAGAGGGG CCCTGCCCCT CCGCCTGCAG CCGTGAAAGG 2040
AAGCTGGGTC ATCAGCCCAG CCCCGCCCAC CCCAGCCCCT ATGTGTGTTT CCCTCAATAA 2100
GGAGATGCCT TGTTCTTTTC ACCATGC 2127

(3) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 513 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS:

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Thr Leu Leu Pro Gly Asp Asn Ser Asp Tyr Asp Tyr Ser Ala
5 10 15
Leu Ser Cys Thr Ser Asp Ala Ser Phe His Pro Ala Phe Leu Pro
20 25 30
Gln Arg Gln Ala Ile Lys Gly Ala Phe Tyr Arg Arg Ala Gln Arg
35 40 45
Leu Arg Pro Gln Asp Glu Pro Arg Gln Gly Cys Gln Pro Glu Asp
50 55 60
Arg Arg Arg Arg Ile Ile Ile Asn Val Gly Gly Ile Lys Tyr Ser

65 70 75

Leu Pro Trp Thr Thr Leu Asp Glu Phe Pro Leu Thr Arg Leu Gly

80 85 90

Gln Leu Lys Ala Cys Thr Asn Phe Asp Asp Ile Leu Asn Val Cys

95 100 105

Asp Asp Tyr Asp Val Thr Cys Asn Glu Phe Phe Phe Asp Arg Asn

110 115 120

Pro Gly Ala Phe Gly Thr Ile Leu Thr Phe Leu Arg Ala Gly Lys

125 130 135

Leu Arg Leu Leu Arg Glu Met Cys Ala Leu Ser Phe Gln Glu Glu

140 145 150

Leu Leu Tyr Trp Gly Ile Ala Glu Asp His Leu Asp Gly Cys Cys

155 160 165

Lys Arg Arg Tyr Leu Gln Lys Ile Glu Glu Phe Ala Glu Met Val

170 175 180

Glu Arg Glu Glu Glu Asp Asp Ala Leu Asp Ser Glu Gly Arg Asp

185 190 195

Ser Glu Gly Pro Ala Glu Gly Glu Gly Arg Leu Gly Arg Cys Met

200 205 210

Arg Arg Leu Arg Asp Met Val Glu Arg Pro His Ser Gly Leu Pro

215 220 225

Gly Lys Val Phe Ala Cys Leu Ser Val Leu Phe Val Thr Val Thr

230 235 240

Ala Val Asn Leu Ser Val Ser Thr Leu Pro Ser Leu Arg Glu Glu

245 250 255

Glu Glu Gln Gly His Cys Ser Gln Met Cys His Asn Val Phe Il

260 265 270

Val Glu Ser Val Cys Val Gly Trp Phe Ser Leu Glu Phe Leu Leu

275 280 285

Arg Leu Ile Gln Ala Pro Ser Lys Phe Ala Phe Leu Arg Ser Pro

290 295 300

Leu Thr Leu Ile Asp Leu Val Ala Ile Leu Pro Tyr Tyr Ile Thr

305 310 315

Leu Leu Val Asp Gly Ala Ala Ala Gly Arg Arg Lys Pro Gly Ala

320 325 330

Gly Asn Ser Tyr Leu Asp Lys Val Gly Leu Val Leu Arg Pal Leu
335 340 345

Arg Ala L u Arg Ile Leu Tyr Val Met Arg Leu Ala Arg His Ser
350 355 360

Leu Gly Leu Gln Thr Leu Gly Leu Thr Ala Arg Arg Cys Thr Arg
365 370 375

Glu Phe Gly Leu Leu Leu Leu Phe Leu Cys Val Ala Ile Ala Leu
380 385 390

Phe Ala Pro Leu Leu Tyr Val Ile Glu Asn Glu Met Ala Asp Ser
395 400 405

Pro Glu Phe Thr Ser Ile Pro Ala Cys Tyr Trp Trp Ala Val Ile
410 415 420

Thr Met Thr Thr Val Asp Tyr Gly Asp Met Val Pro Arg Ser Thr
425 430 435

Pro Gly Gln Val Val Ala Leu Ser Ser Ile Leu Ser Gly Ile Leu
440 445 450

Leu Met Ala Phe Pro Val Thr Ser Ile Phe His Thr Pat Ser Pro
455 460 465

Ser Tyr Leu Glu Leu Lys Gln Glu Gln Glu Arg Val Met Phe Arg
470 475 480

Arg Ala Gln Phe Leu Ile Lys Thr Lys Ser Gln Leu Ser Val Ser
485 490 495

Gln Asp Ser Asp Ile Leu Phe Gly Ser Ala Ser Ser Asp Thr Arg
500 505 510

Asp Asn Asn

(4) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 2483 BASE PAIRS

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
GGTCGCAACC CCTCGGTGAC CCGCTGCGCC CGAGGAGGGG CCGGCGGTGC GCGGTGGTGG    60
CGGCGGGCGC GGCAGCTGTG CCCGTCTGCC CAAGGGTTAA TCCGTCCCCT GCAGCTGCCG   120
CGCGTGCCTT GCAGAATTTC ACCAGAAGAG GGTACAGTTT GAAAAGCTCC TGACGTCAGG   180
CTGGAATTCC TATTGTGTTT AGAAAAGGCT CGGGCAAAGC CAGCCCAAGT TCGCTCTCTG   240
CACACCTCGA GCACCTCGCG GACGGCGTGG GTCCGCCAGC TCCGGGACCT GCCGCCGCTG   300
CCTGCGCGCC CCGGGGCGGA GGACGGTGCC AGCCGCCCAC GAGGAGACCC CGCTCCCGCA   360
GGAGGCCGAG CTGAAGCGGC GGAGCGCGCC GCCAGCCAGC CGGGGTGAGT GCCCCGGGCG   420
AGGCCGGCGG CCGCCAAAGC CCCCGCGGGT TCGTCCGGGC GCCCGGATGC CAGCCCCGAG   480
CCCCGCCGCC GGGTGCATGC CTCCCCCGCG GCGCGCCCCC GCAGGCTGCT GCCCGCTGTG   540
ACCGCCCTTC CCCGCAGGCG GGCGCCGGCC AGGCTCTCCC ACGAGATACG ACGCACGGGT   600
GGCACCCGCC GGACCCCCAA CGACAACGGC GGCGACGTCT GCAGGGGGCG CGGGGCGGAG   660
CCTGCGAGGG CGCGCACGGG GAGGATGGAC GGGTCCGGGG AGCGCAGCCT CCCGGAGCCG   720
GGCAGCCAGA GCTCCGCTGC CAGCGACGAC ATAGAGATAG TCGTCAACGT GGGGGGCGTG   780
CGGCAGGTGC TGTACGGGGA CCTCCTCAGT CAGTACCCTG AGACCCGGCT GGCGGAGCTC   840
ATCAACTGCT TGGCTGGGGG CTACGACACC ATCTTCTCCC TGTGCGACGA CTACGACCCC   900
GGCAAGCGCG AGTTCTACTT TGACAGGGAC CCGGACGCCT TCAAGTGTGT CATCGAGGTG   960
TACTATTTCG GGGAGGTCCA CATGAAGAAG GGCATCTGCC CCATCTGCTT CAAGAACGAG  1020
ATGGACTTCT GGAAGGTGGA CCTCAAGTTC CTGGACGACT GTTGCAAGAG CCACCTGAGC  1080
GAGAAGCGCG AGGAGCTGGA GGAGATCGCG CGCCGCGTGC AGCTCATCCT GGACGACCTG  1140
GGCGTGGACG CGGCCGAGGG CCGCTGGCGC CGCTGCCAGA AGTGCGTCTG GAAGTTCCTG  1200
GAGAAGCCCG AGTCGTCGTG CCCGGCGCGG GTGGTGGCCG AGCTCTCCTT CCTGCTCATC  1260
CTCGTCTCGT CCGTGGTCAT GTGCATGGAC ACCATCCCCG AACTGCAGGT GCTGGACGCC  1320
GAGGGCAACC GCGTGGAGCA CCCGACGCTG GAGAACGTGG AGACGGCGTG CATTGGCTGG  1380
TTCACCCTGG AGTACCTGCT GCGCCTCTTC TCGTCACCCA ACAAGCTGCA CTTCGCGCTG  1440
TCCTTCATGA ACATTGTGGA CGTGCTGGCC ATCCTCCCCT TCTACGTGAG CCTCACGCTC  1500
ACGCACCTGG GTGCCCGCAT GATGGAGCTG ACCAACGTGC AGCAGGCCGT GCAGGCGCTG  1560
CGGATCATGC GCATCGCGCG CATCTTCAAG CTGGCCCGCC ACTCCTCGGG CCTGCAGACC  1620
CTCACCTATG CCCTCAAGCG CAGCTTCAAG GAACTGGGGC TGCTGCTCAT GTACCTGGCA  1680
GTGGGTATCT TCGTCTTCTC TGCCCTGGGC TACACCATGG AGCAGAGCCA TCCAGAGACC  1740
CTGTTTAAGA ACATCCCCCA GTCCTTCTGG TGGGCCATCA TCACCATGAC CACCGTCGGC  1800
TACGGCGACA TCTACCCCAA GACCACGCTG AGCAAGCTCA ACGCGGCCAT CAGCTTCTTG  1860
TGTGGTGTCA TTGCCATCGC CCTGCCCATC CACCCCATCA TCAACAACTT TGTCAGGTAC  1920
TACAACAAGC AGCGCGTCCT GGAGACCGCG GCCAAGCACG AGCTGGAGCT GATGGAACTC  1980
AACTCCAGCA GCGGGGGCGA GGGCAAGACC GGGGGCTCCC GCAGTGACCT GGACAACCTC  2040
CCTCCAGAGC CTGCGGGGAA GGAGGCGCCG AGCTGCAGCA GCCGGCTGAA GCTCTCCCAC  2100
AGCGACACCT TCATCCCCCT CCTGACCGAG GAGAAGCACC ACAGGACCCG GCTCCAGAGT  2160
TGCAAGTGAC AGGAGGCCCC TCAGGCAGAG ATGGACCAGG CGGTGGACAG ATGGGTAGAT  2220
GTGGCAGGCA TGTCATCGAC AGCACAGAAG GGCTGTCCTG TGTCCCCCCA ACCCTCCCCT  2280
GGACAGACTC TGAAGGCCCT CCCGGCACCT CTGCCAAGGC TGGGTAAGAC TCCTCTATGT  2340
TGCCTGCTGT CCAGGAGCCC GGGAGGGAGG GGTGTGCAGG AGCCGCAGGG CCGTGTGGGA  2400
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CGAGTGGAGG CCGCGGCCTG GCTGGCACGA GAGCCCACGC CCGCTTCTGT ATCTCCCTCA 2460
ATAAAGCCTC CTGCTCTGTG CAA 2483

(5) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 494 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS:

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Asp Gly Ser Gly Glu Arg Ser Leu Pro Glu Pro Gly Ser Gln
5 10 15
Ser Ser Ala Ala Ser Asp Asp Ile Glu Ile Val Val Asn Val Gly
20 25 30
Gly Val Arg Gln Val Leu Tyr Gly Asp Leu Leu Ser Gln Tyr Pro
35 40 45
Glu Thr Arg Leu Ala Glu Leu Ile Asn Cys Leu Ala Gly Gly Tyr
50 55 60
Asp Thr Ile Phe Ser Leu Cys Asp Asp Tyr Asp Pro Gly Lys Arg
65 70 75
Glu Phe Tyr Phe Asp Arg Asp Pro Asp Ala Phe Lys Cys Val Ile
80 85 90
Glu Val Tyr Tyr Phe Gly Glu Val His Met Lys Lys Gly Ile Cys
95 100 105
Pro Ile Cys Phe Lys Asn Glu Met Asp Phe Trp Lys Val Asp Leu
110 115 120
Lys Phe Leu Asp Asp Cys Cys Lys Ser His Leu Ser Glu Lys Arg
125 130 135
Glu Glu Leu Glu Glu Ile Ala Arg Arg Val Gln Leu Ile Leu Asp
140 145 150
Asp Leu Gly Val Asp Ala Ala Glu Gly Arg Trp Arg Arg Cys Gln

155 160 165

Lys Cys Val Trp Lys Phe Leu Glu Lys Pro Glu Ser Ser Cys Pro

170 175 180

Ala Arg Val Val Ala Glu Leu Ser Phe Leu Leu Ile Leu Val Ser

185 190 195

Ser Val Val Met Cys Met Asp Thr Ile Pro Glu Leu Gln Val Leu

200 205 210

Asp Ala Glu Gly Asn Arg Val Glu His Pro Thr Leu Glu Asn Val

215 220 225

Glu Thr Ala Cys Ile Gly Trp Phe Thr Leu Glu Tyr Leu Leu Arg

230 235 240

Leu Phe Ser Ser Pro Asn Lys Leu His Phe Ala Leu Ser Phe Met

245 250 255

Asn Ile Val Asp Val Leu Ala Ile Leu Pro Phe Tyr Val Ser Leu

260 265 270

Thr Leu Thr His Leu Gly Ala Arg Met Met Glu Leu Thr Asn Val

275 280 285

Gln Gln Ala Val Gln Ala Leu Arg Ile Met Arg Ile Ala Arg Il

290 295 300

Phe Lys Leu Ala Arg His Ser Ser Gly Leu Gln Thr Leu Thr Tyr

305 310 315

Ala Leu Lys Arg Ser Phe Lys Glu Leu Gly Leu Leu Leu Met Tyr

320 325 330

Leu Ala Val Gly Ile Phe Val Phe Ser Ala Leu Gly Tyr Thr Met

335 340 345

Glu Gln Ser His Pro Glu Thr Leu Phe Lys Asn Ile Pro Gln Ser

350 355 360

Phe Trp Trp Ala Ile Ile Thr Met Thr Thr Val Gly Tyr Gly Asp

365 370 375

Ile Tyr Pro Lys Thr Thr Leu Ser Lys Leu Asn Ala Ala Ile Ser

380 385 390

Phe Leu Cys Gly Val Ile Ala Ile Ala Leu Pro Ile His Pro Il

395 400 405

Ile Asn Asn Phe Val Arg Tyr Tyr Asn Lys Gln Arg Val Leu Glu

410 415 420

Thr Ala Ala Lys His Glu Leu Glu Leu Met Glu Leu Asn Ser Ser
425 430 435

Ser Gly Gly Glu Gly Lys Thr Gly Gly Ser Arg Ser Asp Leu Asp
440 445 450

Asn Leu Pro Pro Glu Pro Ala Gly Lys Glu Ala Pro Ser Cys Ser
455 460 465

Ser Arg Leu Lys Leu Ser His Ser Asp Thr Phe Ile Pro Leu Leu
470 475 480

Thr Glu Glu Lys His His Arg Thr Arg Leu Gln Ser Cys Lys
485 490

WHAT IS CLAIMED IS:

1. An isolated polynucleotide s l cted from th group consisting of

(a) a polynucleotide encoding a K^+ channel 1 polypeptide having the deduced amino acid sequence of Figure 1 or a fragment, analog or derivative of said polypeptide;

(b) a polynucleotide encoding a K^+ channel 2 polypeptide having the deduced amino acid sequence of Figure 2 or a fragment, analog or derivative of said polypeptide;

(c) a polynucleotide encoding a K^+ channel 1 polypeptide having amino acid sequence encoded by the cDNA contained in ATCC Deposit No. 75700 or a fragment, analog or derivative of said polypeptide; and

(d) a polynucleotide encoding a K^+ channel 2 polypeptide having the amino acid sequence encoded by the cDNA contained in ATCC Deposit No. 75830 or a fragment, analog or derivative of said polypeptide.

2. The polynucleotide of Claim 1 wherein the polynucleotide is DNA.

3. The polynucleotide of Claim 1 wherein the polynucleotide is RNA.

4. The polynucleotide of Claim 1 wherein the polynucleotide is genomic DNA.

5. The polynucleotide of Claim 2 wherein said polynucleotide encodes a K^+ channel 1 polypeptide having the deduced amino acid sequence of Figures 1 and 2.

6. The polynucleotide of Claim 2 wherein said polynucleotide encodes a K^+ channel 1 polypeptide encoded by the cDNA of ATCC Deposit No. 75700.

7. Th polynucleotide of Claim 2 wherein said polynucleotide encodes a K^+ channel 2 polypeptide having the deduced amino acid sequence of Figure 2.

8. The polynucleotide of Claim 2 having the coding sequence of a K^+ channel 2 polypeptide deposited as ATCC Deposit No. 75830.

9. A vector containing the DNA of Claim 2.

10. A host cell genetically engineered with the vector of Claim 9.

11. A process for producing a polypeptide comprising: expressing from the host cell of Claim 10 the polypeptide encoded by said DNA.

12. A process for producing cells capable of expressing a polypeptide comprising genetically engineering cells with the vector of Claim 9.

13. An isolated DNA hybridizable to the DNA of Claim 2 and encoding a polypeptide having K^+ channel 1 polypeptide activity.

14. An isolated DNA hybridizable to the DNA of Claim 2 and encoding a polypeptide having K^+ channel 2 polypeptide activity.

15. A polypeptide selected from the group consisting of (i) a K^+ channel 1 polypeptide having the deduced amino acid sequence of Figure 1 and fragments, analogs and derivatives thereof; (ii) a K^+ channel 2 polypeptide having the deduced amino acid sequence of Figure 2 and fragments, analogs and derivatives thereof; (iii) a K^+ channel 1 polypeptide encoded by the cDNA of ATCC Deposit No. 75700 and fragments, analogs and derivatives of said polypeptide; and (iv) a K^+ channel 2 polypeptide encoded by the cDNA of ATCC Deposit No. 75830 and fragments, analogs and derivatives of said polypeptide.

16. The polypeptide of claim 15 wherein the polyp ptide is a K^+ channel 1 polypeptide having the deduced amino acid sequence of Figure 1.

17. The polypeptide of claim 15 wherein the polypeptide is a K^+ channel 2 polypeptide having the deduced amino acid sequence of Figure 2.

18. Antibodies against the polypeptide of claim 15.

19. Agonists for the polypeptide of claim 15.

20. Antagonists against the polypeptide of claim 15.

21. A method for the treatment of a patient having need of an agonist to a K^+ channel 1 polypeptide comprising: administering to the patient a therapeutically effective amount of the agonist of claim 19.

22. A method for the treatment of a patient having need of an agonist to a K^+ channel 2 polypeptide comprising: administering to the patient a therapeutically effective amount of the agonist of claim 19.

23. A method for the treatment of a patient having need to inhibit a K^+ channel 1 polypeptide comprising: administering to the patient a therapeutically effective amount of an antagonist/inhibitor of claim 20.

24. A method for the treatment of a patient having need to inhibit a K^+ channel 2 polypeptide comprising: administering to the patient a therapeutically effective amount of an antagonist of claim 20.

25. A process for identifying molecules having a modulating effect on K^+ Channel expression which comprises:

providing an expression system that produces a functional K^+ channel expression product of a K^+ channel gene;

contacting said product with one or more molecules to determine its modulating effect on the bioactivity of said product; and

selecting fr m said molecul s a candidate capable of modulating said K^+ channel expression.

FIG. 1A

```
ACAAAAGCTGGAGCTCCACCGCGGTGCGGCCGCTCTAGAACTAGTGGATCCCCCGGGCTG
CAGGGGCTCCGAGGGCGGGAGCTGAGCCGGGCCCCGGACCGAAGTTTGGCGGCGGCTCC
GGGAGGCAGAGCGGGCTCCCCGGGCGACTTCCAGGCCCCTCTCGCGTCCTCGCCCCGGAC
CCGTGGGCAGTCGGGGGGACGGAAGCCGCGGCCGGGCCAACTCCGAGGCGGGGACGCCG
CGACGGGAACTTGAGGCCCGAGAGGGATGTGAAGGCCCAAAATGACCCTCTTACCGGGAG
                                              M  T  L  L  P  G  D
ACAATTCTGACTACGACTACAGCGCGCTGAGCTGCACCTCGGACGCCTCCTTCCACCCGG
 N  S  D  Y  D  Y  S  A  L  S  C  T  S  D  A  S  F  H  P  A
CCTTCCTCCCGCAGCGCCAGGCCATCAAGGGCGCGTTCTACCGCCGGGCGCAGCGGCTGC
 F  L  P  Q  R  Q  A  I  K  G  A  F  Y  R  R  A  Q  R  L  R
GGCCGCAGGATGAGCCCCGCCAGGGCTGTCAGCCCGAGGACCGCCGCCGTCGGATCATCA
 P  Q  D  E  P  R  Q  G  C  Q  P  E  D  R  R  R  R  I  I  I
TCAACGTAGGCGGCATCAAGTACTCGCTGCCCTGGACCACGCTGGACGAGTTCCCGCTGA
 N  V  G  G  I  K  Y  S  L  P  W  T  T  L  D  E  F  P  L  T
CGCGCCTGGGCCAGCTCAAGGCCTGCACCAACTTCGACGACATCCTCAACGTGTGCGATG
 R  L  G  Q  L  K  A  C  T  N  F  D  D  I  L  N  V  C  D  D
ACTACGACGTCACCTGCAACGAGTTCTTCTTCGACCGCAACCCGGGGGCCTTCGGCACTA
 Y  D  V  T  C  N  E  F  F  F  D  R  N  P  G  A  F  G  T  I
TCCTGACCTTCCTGCGCGCGGGCAAGCTGCGGCTGCTGCGCGAGATGTGCGCGCTGTCCT
 L  T  F  L  R  A  G  K  L  R  L  L  R  E  M  C  A  L  S  F
TCCAGGAGGAGCTGCTGTACTGGGGCATCGCGGAGGACCACCTGGACGGCTGCTGCAAGC
 Q  E  E  L  L  Y  W  G  I  A  E  D  H  L  D  G  C  C  K  R
GCCGCTACCTGCAGAAGATTGAGGAGTTCGCGGAGATGGTGGAGCGGGAGGAAGAGGACG
 R  Y  L  Q  K  I  E  E  F  A  E  M  V  E  R  E  E  E  D  D
ACGCGCTGGACAGCGAGGGCCGCGACAGCGAGGGCCCGGCCGAGGGCGAGGGCCGCCTGG
 A  L  D  S  E  G  R  D  S  E  G  P  A  E  G  E  G  R  L  G
GGCGCTGCATGCGGCGACTGCGCGACATGGTGGAGAGGCCGCACTCGGGGCTGCCTGGCA
 R  C  M  R  R  L  R  D  M  V  E  R  P  H  S  G  L  P  G  K
AGGTGTTCGCCTGCCTGTCGGTGCTCTTCGTGACCGTCACCGCCGTCAACCTCTCCGTCA
```

MATCH WITH FIG. 1B

MATCH WITH FIG. IA

FIG. 1B

```
 V  F  A  C  L  S  V  L  F  V  T  V  T  A  V  N  L  S  V  S
GCACCTTGCCCAGCCTGAGGGAGGAGGAGGAGCAGGGCCACTGTTCCCAGATGTGCCACA
 T  L  P  S  L  R  E  E  E  E  Q  G  H  C  S  Q  M  C  H  N
ACGTCTTCATCGTGGAGTCGGTGTGCGTGGGCTGGTTCTCCCTGGAGTTCCTCCTGCGGC
 V  F  I  V  E  S  V  C  V  G  W  F  S  L  E  F  L  L  R  L
TCATTCAGGCGCCCAGCAAGTTCGCCTTCCTGCGGAGCCCGCTGACGCTGATCGACCTGG
 I  Q  A  P  S  K  F  A  F  L  R  S  P  L  T  L  I  D  L  V
TGGCCATCCTGCCCTACTACATCACGCTGCTGGTGGACGGCGCCGCCGCAGGCCGTCGCA
 A  I  L  P  Y  Y  I  T  L  L  V  D  G  A  A  A  G  R  R  K
AGCCCGGCGCGGGCAACAGCTACCTGGACAAGGTGGGGCTGGTGCTGCGCGTGCTGCGGG
 P  G  A  G  N  S  Y  L  D  K  V  G  L  V  L  R  V  L  R  A
CGCTGCGCATCCTGTACGTGATGCGCCTGGCGCGCCACTCCCTGGGGCTGCAGACGCTGG
 L  R  I  L  Y  V  M  R  L  A  R  H  S  L  G  L  Q  T  L  G
GGCTCACGGCCCGCCGCTGCACCCGCGAGTTCGGGCTCCTGCTGCTCTTCCTCTGCGTGG
 L  T  A  R  R  C  T  R  E  F  G  L  L  L  L  F  L  C  V  A
CCATCGCCCTCTTCGCGCCCCTGCTCTACGTCATCGAGAACGAGATGGCCGACAGCCCCG
 I  A  L  F  A  P  L  L  Y  V  I  E  N  E  M  A  D  S  P  E
AGTTCACCAGCATCCCTGCCTGCTACTGGTGGGCTGTCATCACCATGACGACGGTGGACT
 F  T  S  I  P  A  C  Y  W  W  A  V  I  T  M  T  T  V  D  Y
ATGGCGACATGGTCCCCAGGAGCACCCCGGGCCAGGTAGTGGCCCTGAGCAGCATCCTGA
 G  D  M  V  P  R  S  T  P  G  Q  V  V  A  L  S  S  I  L  S
GCGGCATCCTGCTCATGGCCTTCCCAGTCACCTCCATCTTCCACACCTTCTCCCCCTCCT
 G  I  L  L  M  A  F  P  V  T  S  I  F  H  T  F  S  P  S  Y
ACCTGGAGCTCAAACAGGAGCAAGAGAGGGTGATGTTCCGGAGGGCGCAGTTCCTCATCA
 L  E  L  K  Q  E  Q  E  R  V  M  F  R  R  A  Q  F  L  I  K
AAACCAAGTCGCAGCTGAGCGTGTCCCAGGACAGTGACATCTTGTTCGGAAGTGCCTCCT
 T  K  S  Q  L  S  V  S  Q  D  S  D  I  L  F  G  S  A  S  S
CGGACACCAGAGACAATAACTGAGCGCGGAGGACACGCCTGCCCTGCCTGCCATCTGTGG
 D  T  R  D  N  N  *
```

MATCH WITH FIG. IC

MATCH WITH FIG. 1B

FIG. 1C

CCCGAAGCCATTGCCATCCACTGCAGACGCCTGGAGAGGGACAGGCCGCTTCCGAGTGCA
GTCCTGGCGCAGCACCGACTCCCACGCACCCGGGGAAGGACACCCTCACTCCCACACCCC
GGGAAGAACACTAGAACATCAGCAGAGGGGCCCTGCCCCTCCGCCTGCAGCCGTGAAAGG
AAGCTGGGTCATCAGCCCAGCCCCGCCCACCCCAGCCCCTATGTGTGTTTCCCTCAATAA
GGAGATGCCTTGTTCTTTTCACCATGC

FIG. 2A

```
        10                       30                       50
GGTCGCAACCCCTCGGTGACCCGCTGCGCCCGAGGAGGGGCCGGCGGTGCGCGGTGGTGG
        70                       90                      110
CGGCGGGCGCGGCAGCTGTGCCCGTCTGCCCAAGGGTTAATCCGTCCCCTGCAGCTGCCG
       130                      150                      170
CGCGTGCCTTGCAGAATTTCACCAGAAGAGGGTACAGTTTGAAAAGCTCCTGACGTCAGG
       190                      210                      230
CTGGAATTCCTATTGTGTTTAGAAAAGGCTCGGGCAAAGCCAGCCCAAGTTCGCTCTCTG
       250                      270                      290
CACACCTCGAGCACCTCGCGGACGGCGTGGGTCCGCCAGCTCCGGGACCTGCCGCCGCTG
       310                      330                      350
CCTGCGCGCCCCGGGGCGGAGGACGGTGCCAGCCGCCCACGAGGAGACCCCGCTCCCGCA
       370                      390                      410
GGAGGCCGAGCTGAAGCGGCGGAGCGCGCCGCCAGCCAGCCGGGGTGAGTGCCCCGGGCG
       430                      450                      470
AGGCCGGCGGCCGCCAAAGCCCCCGCGGGTTCGTCCGGGCGCCCGGATGCCAGCCCCGAG
       490                      510                      530
CCCCGCCGCCGGGTGCATGCCTCCCCCGCGGCGCGCCCCCGCAGGCTGCTGCCCGCTGTG
       550                      570                      590
```

MATCH WITH FIG. 2B

MATCH WITH FIG. 2A

FIG. 2B

```
ACCGCCCTTCCCCGCAGGCGGGCGCCGGGCAGGCTCTCCCACGAGATACGACGCACGGGT
    610                         630                         650
GGCACCCGCCGGACCCCCAACGACAACGGCGGCGACGTCTGCAGGGGGCGCGGGGCGGAG
    670                         690                         710
CCTGCGAGGGCGCGCACGGGGAGGATGGACGGTCCGGGGAGCGCAGCCTCCCGGAGCCG
                          M  D  G  S  G  E  R  S  L  P  E  P
    730                         750                         770
GGCAGCCAGAGCTCCGCTGCCAGCGACGACATAGAGATAGTCGTCAACGTGGGGGGCGTG
G  S  Q  S  S  A  A  S  D  D  I  E  I  V  V  N  V  G  G  V
    790                         810                         830
CGGCAGGTGCTGTACGGGGACCTCCTCAGTCAGTACCCTGAGACCCGGCTGGCGGAGCTC
R  Q  V  L  Y  G  D  L  L  S  Q  Y  P  E  T  R  L  A  E  L
    850                         870                         890
ATCAACTGCTTGGCTGGGGGCTACGACACCATCTTCTCCCTGTGCGACGACTACGACCCC
I  N  C  L  A  G  G  Y  D  T  I  F  S  L  C  D  D  Y  D  P
    910                         930                         950
GGCAAGCGCGAGTTCTACTTTGACAGGGACCCGGACGCCTTCAAGTGTGTCATCGAGGTG
G  K  R  E  F  Y  F  D  R  D  P  D  A  F  K  C  V  I  E  V
    970                         990                        1010
TACTATTTCGGGGAGGTCCACATGAAGAAGGGCATCTGCCCCATCTGCTTCAAGAACGAG
Y  Y  F  G  E  V  H  M  K  K  G  I  C  P  I  C  F  K  N  E
   1030                        1050                        1070
ATGGACTTCTGGAAGGTGGACCTCAAGTTCCTGGACGACTGTTGCAAGAGCCACCTGAGC
M  D  F  W  K  V  D  L  K  F  L  D  D  C  C  K  S  H  L  S
   1090                        1110                        1130
GAGAAGCGCGAGGAGCTGGAGGAGATCGCGCGCCGCGTGCAGCTCATCCtgGACGACCTG
```

MATCH WITH FIG. 2C

FIG. 2C

MATCH WITH FIG. 2B

```
E  K  R  E  E  L  E  E  I  A  R  R  V  Q  L  I  L  D  D  L
      1150                   1170                   1190
GGCGTGGACGCGGCCGAGGGCCGCTGGCGCCGCTGCCAGATGTGCGTCTGGAAGTTCCTG
G  V  D  A  A  E  G  R  W  R  R  C  Q  K  C  V  W  K  F  L
      1210                   1230                   1250
GAGAAGCCCGAGTCGTCGTGCCCGGCGCGGGTGGTGGCCGAGCTCTCCTTCCTGCTCATC
E  K  P  E  S  S  C  P  A  R  V  V  A  E  L  S  F  L  L  I
      1270                   1290                   1310
CTCGTCTCGTCCGTGGTCATGTGCATGGACACCATCCCCGAACTGCAGGTGCTGGACGCC
L  V  S  S  V  V  M  C  M  D  T  I  P  E  L  Q  V  L  D  A
      1330                   1350                   1370
GAGGGCAACCGCGTGGAGCACCCGACGCTGGAGAACGTGGAGACGGCGTGCATTGGCTGG
E  G  N  R  V  E  H  P  T  L  E  N  V  E  T  A  C  I  G  W
      1390                   1410                   1430
TTCACCCTGGAGTACCTGCTGCGCCTCTTCTCGTCACCCAACAAGCTGCACTTCGCGCTG
F  T  L  E  Y  L  L  R  L  F  S  S  P  N  K  L  H  F  A  L
      1450                   1470                   1490
TCCTTCATGAACATTGTGGACGTGCTGGCCATCTCTCCCTTCTACGTGAgCTCTACGcTC
S  F  M  N  I  V  V  V  L  A  I  L  P  F  Y  V  S  L  T  L
      1510                   1530                   1550
ACGCACCTGGGTGCCCGCATGATGGAGCTGACCAACGTGCAGCAGGCCGTGCAGGCGCTG
T  H  L  G  A  R  M  M  E  L  T  N  V  Q  Q  A  V  Q  A  L
      1570                   1590                   1610
CGGATCATGCGCATCGCGCGCATCTTCAAGCTGGCCCGCCACTCCTCGGGCCTGCAGACC
R  I  M  R  I  A  R  I  F  K  L  A  R  H  S  S  G  L  Q  L
      1630                   1650                   1670
```

MATCH WITH FIG. 2D

FIG. 2D

MATCH WITH FIG. 2C

```
CTCACCTATGCCCTCAAGCGCAGCTTCAAGGAACTGGGGCTGCTGCTCATGTACCTGGCA
L  T  Y  A  L  K  R  S  F  K  E  L  G  L  L  L  M  Y  L  A
    1690                          1710                          1730
GTGGGTATCTTCGTCTTCTCTGCCCTGGGCTACACCATGGAGCAGAGCCATCCAGAGACC
V  G  I  F  V  F  S  A  L  G  Y  T  M  E  Q  S  H  P  E  T
    1750                          1770                          1790
CTGTTTAAGAACATCCCCCAGTCCTTCTGGTGGGCCATCATCACCATGACCACCGTCGGC
L  F  K  N  I  P  Q  S  F  W  W  A  I  I  T  M  T  T  V  G
    1810                          1830                          1850
TACGGCGACATCTACCCCAAGACCACGCTGAGCAAGCTCAACGCGGCCATCAGCTTCTTG
Y  G  D  I  Y  P  K  T  T  L  S  K  L  N  A  A  I  S  F  L
    1870                          1890                          1910
TGTGGTGTCATTGCCATCGCCCTGCCCATCCACCCCATCATCAACAACTTTGTCAGGTAC
C  G  V  I  A  I  A  L  P  I  H  P  I  I  N  N  F  V  R  Y
    1930                          1950                          1970
TACAACAAGCAGCGCGTCCTGGAGACCGCGGCCAAGCACGAGCTGGAGCTGATGGAACTC
Y  N  K  Q  R  V  L  E  T  A  A  K  H  E  L  E  L  M  E  L
    1990                          2010                          2030
AACTCCAGCAGCGGGGGCGAGGGCAAGACCGGGGGCTCCCGCAGTGACCTGGACAACCTC
N  S  S  S  G  G  E  G  K  T  G  G  S  R  S  D  L  D  N  L
    2050                          2070                          2090
CCTCCAGAGCCTGCGGGGAAGGAGGCGCCGAGCTGCAGCAGCCGGCTGAAGCTCTCCCAC
P  P  E  P  A  G  K  E  A  P  S  C  S  S  R  L  K  L  S  H
    2110                          2130                          2150
AGCGACACCTTCATCCCCCTCCTGACCGAGGAGAAGCACCACAGGACCCGGCTCCAGAGT
S  D  T  F  I  P  L  L  T  E  E  K  H  H  R  T  R  L  Q  S
```

MATCH WITH FIG. 2E

MATCH WITH FIG. 2D

FIG. 2E

```
          2170                     2190                      2210
TGCAAGTGACAGGAGGCCCCTCAGGCAGAGATGGACCAGGCGGTGGACAGATGGGTAGAT
C  K  *
          2230                     2250                      2270
GTGGCAGGCATGTCATCGACAGCACAGAAGGGCTGTCCTGTGTCCCCCAACCCTCCCCT
          2290                     2310                      2330
GGACAGACTCTGAAGGCCTCTCCGGCACCTTCGCCAAGGCTGGGTAAGACTCCTCTATGT
          2350                     2370                      2390
TGCCTGCTGTCCAGGAGCCCGGGAGGGAGGGGTGTGCAGGAGCCGCAGGGCCGTGTGGGA
          2410                     2430                      2450
CGAGTGGAGGCCGCGGCCTGGCTGGCACGAGAGCCCACGCCCGCTTCTGTATCTCCCTCA
          2470
ATAAAGCCTCCTGCTCTGTGCAA
```

FIG. 3A

```
 4  SGERSLPEPGSOSSAASDDIEIV..........VNVGGVRQVLYGDLLSQ  43

 2  PAGMTKHGSRSTSSLPPEPMEIVRSKACSPRVRLNVGGLAHEVLWRTLDR  51

44  YPETRLAELINCLAGGYDTIFSBCDDYDPGKREFYFDRDPDAFKCVIEVY  93

52  LPRTRLGKLRDC..NTHDSLLEVCDDYSLDDNEYFFDRHPGAFTSILNFY  99

94  YFGEVHMKKGICPICFRNEMDFWKVDLKFLDDCCKSHLSEKREELEEIAR 143
```

MATCH WITH FIG. 3B

MATCH WITH FIG. 3A

FIG. 3B

```
100  RTGRLHMMEEMCALSFSQELDYWGIDEIYLESCCQARYHQKKEQMNEELK  149
141  RVQLIL.....DDLGVDAAEGRWRRCQKCVWKFLEKPESSCPARVVAELS  188
150  REAETLREREGEEFDNTCCAEKRKK....LWDLLEKPNSSVAAKILAIIS  195
189  FLLILVSSVVMCMDTIPELQVLDAEGNRVEHPTLENVETACIGWFTLEYL  238
196  IMFIVLSTIALSLNTLPELQSLDEFGQSTDNPQLAHVEAVCIAWFTMEYL  245
239  LRLFSSPNKLHFALSFMNIVDVLAILPFYVSLTLTHLGARMMELTNVQQA  288
246  LRFLSSPKKWKFFKGPLNAIDLLAILPYYVTIFLTESNKSVLQFQNVRRV  295
289  VQALRIMRIARIFKLARHSSGLQTLTYALKRSFKELGLLLMYLAVGIFIF  338
296  VQIFRIMRILRILKLARHSTGLQSLGFTLRRSYNELGLLILFLAMGIMIF  345
339  SALGYTMEQSHPETLFKNIPQSFWWAIITMTTVGYGDIYPKTTLSKLNAA  388
```

MATCH WITH FIG. 3C

MATCH WITH FIG. 3B

FIG. 3C

```
346 SSLVFFAEKDEDDTKFKSIPASFWWATITMTTVGYGDIYPKTLLGKIVGG 395

389 ISFLCGVIAIALPIHPIINNFVRYYNKQRVLETAAK.............. 424

396 LCCIAGVLVIALPIPIIVNNFSEFYKEQKRQEKAIKRREALERAKRNGSI 445

425 ..........HELELMELNSSSGGEGKTGGSRSDLDNLPP.......... 454

446 VSMNMKDAFARSIEMMDIVVEKNGENMGKKDKVQDNHLSPNKWKWTKRTL 495

455 .............EPAGKEAPSCSSRLKLSHSDTFIPLLTEEKHHRTRLQ 491

496 SETSSSKSFETKEQGSPEKARSSSSPQHLN.....VQQLEDMYNKMAKTQ 540
492 S 492
    |
541 S 541
```

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/08449

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL : 435/69.1, 240.1, 252.3, 254, 320.1; 530/350; 536/23.5
According to International Patent Classification (IPC) r to both national classificati n and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 240.1, 252.3, 254, 320.1; 530/350; 536/23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Proceedings of the National Academy of Sciences USA, Volume 91, issued April 1994, R. Milkman, "An *Escherichia coli* homologue of eukaryotic potassium channel proteins", pages 3510-3514, entire document.	1-17
A	Annals of the New York Academy of Sciences, Volume 707, issued 1993, M. Li et al., "Assembly of potassium channels", pages 51-59, entire document.	1-17
X	Journal of Neuroscience, Volume 12, Number 2, issued February 1992, J.A. Drewe et al., "Distinct spatial and temporal expression patterns of K+ channel mRNAs from different subfamilies", pages 538-548, entire document.	1-17

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion f the international search 15 NOVEMBER 1994	Date f mailing of the international search report 05 DEC 1994
Name and mailing address f the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile N . (703) 305-3230	Authorized fficer GABRIELE BUGAISKY Telephone N . (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/08449

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 19-25
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Please See Extra Sheet.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-17

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/08449

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C07H 21/00, 21/04; C07K 14/435, 16/18; C12N 1/19, 1/21, 5/10, 15/12, 15/63; C12P 21/02

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, Dialog files 5, 155, 357 (Biosis, Medline, Biotechnology Abstracts), N-Geneseq 16; EMBL-New 9, Genbank 84, Genbank-New 9, UEMBL 39 84, A-Geneseq 16, PIR 41, Swiss-Prot 29
search terms: potassium, channel? , shab, shaker, shaw, shal, drk?, delay? (3n) rectif?, brain, gene? ?, DNA, human,

BOX I. OBSERVATIONS WHERE CLAIMS WERE FOUND UNSEARCHABLE
2. Where no meaningful search could be carried out, specifically:

No agonists, antagonists or treatment methods have been provided for; the specification merely discloses the potential for any of the above. It is not even clear that the disclosed genes encode bona fide K+ channels, as the only evidence supporting such a conclusion is based on sequence similarities; no functional analyses of the translation product have been performed to demonstrate channel activity. Without a showing of the biological role of the translated product, it is impossible to search for agonists, antagonists, etc.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

Group I, claim(s) 1-17, drawn to K+ channel genes, cloned cells, expression method, and expressed protein.
Group II, claim(s) 18, drawn to antibodies directed against channel proteins.
The inventions listed as Groups I and II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: An antibody is encoded by an entirely different DNA than that the protein which is bound by it, and the primary sequence of the antibody bears no relationship to the sequence of the detected protein.